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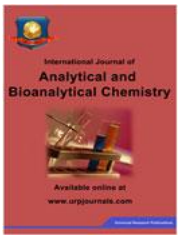

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Review Article

Brief About Impurity Profiling

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Abstract

Impurity is considered as any other organic material, besides the drug substance, or ingredients, arise out of synthesis or unwanted chemicals that remains with API's. Impurity developed during formulation and it is affected on safety and efficacy of pharmaceutical product. Impurity also affected on absorption, distribution, metabolism and elimination of drug in human body. Impurity profile study is needed for biological safety. Impurity profiling includes identification, structure elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulations. In Impurity profile impurity should be detected and determined by selective method. Various chromatographic and spectroscopic methods or techniques are used for impurity profile.

Keywords: Impurity profiling, characterization of impurity, isolation of impurity, types of impurity profiling.

Introduction

Impurity is anything which makes something impure or which effect on quality, safety of drug which is harmful to human or animal body. Impurity is defined as any drug or substances which coincide with the main or original drug, which formed due to any chemical reaction or side effect. We can also define as any material or substance that affects the purity of the material of interest i.e. API or any drug substance. Safety of drug is depending upon toxicological property as well as impurity contains itself. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's).

Impurity profile

Impurity profile describes the identified and unidentified impurities present in a new drug substance. Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. It helps identifying the impurity present in pharmaceutical formulation by analytical technique or methods. Numbers of impurities present in formulation such by-products, degradation products, interaction products, intermediates, penultimate intermediates, related products, Transformation product. According to USP impurities have various sections which are Impurities in Official Articles, Ordinary Impurities, and Organic Volatile Impurities. According to ICH impurities occurred or produced by chemical syntheses which are organic impurities (Process and Drug related), Inorganic Impurities, and Residual Solvents. There are various sources of impurity like heavy metals, ligands, catalysts other materials like degraded end products obtained during or after manufacturing of bulk drug or products. The expert working group of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use commonly known ICH has "defined impurity is any compound of the medicinal product which is not the chemical entity defined as the active substance or as an excipient in the product".

Critical Factors Affects the Quality of Bulk Drugs

1. Crystallization
2. Washing the wet cake
3. Drying
4. Appropriate packaging

Classification[1-8]

1) Dosage form related[2]

i. Mutual interaction amongst Ingredients
ii. Functional group- related typical Degradation

iii. Ester hydrolysis
iv. Hydrolysis
v. Oxidative degradation

2. USP Classification

According to united state pharmacopoeia impurities classify as

i. Impurities in official articles
ii. Ordinary impurities
iii. Organic volatile impurities

3. ICH Classification[2]

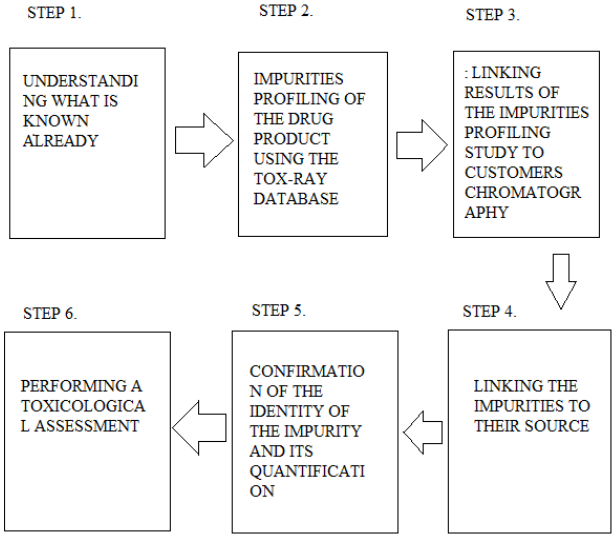
i. Organic impurities
ii. Inorganic impurities
iii. Residual solvents

Impurities coming from

- Interaction between the primary packaging and drug product
- At the time of contact between processing materials and storage bags, closure, filters, tubing material etc.
- Impurities also introduce during storage of compound
- Impurities also coming from label, ink, overwrap, cardboard, boxes etc.
- Impurities can be present at the synthesis of product (called genotoxic impurities) that is solvent, residues, catalysts, reaction product in synthesis etc.

Identification of impurities

Six steps are there for identification of impurities



Method for impurity detection

1. Isolation and characterization
2. Column chromatography
3. Gas chromatography
4. Flash chromatography
5. TLC
6. GC
7. HPLC
8. HPTLC
9. Capillary electrophoresis (CE)

1. Isolation and characterization

Isolation of impurity is necessary for accurate monitor, but it is only necessary if instrumental method is not available as it directly characterizes the impurity. Which method used for isolation and characterization of impurity is depends upon the nature of the impurity that means structure of impurity, its physicochemical properties and availability. For isolation of impurity methods used are, Chromatographic and Non –Chromatographic.

Following methods are commonly used for the isolation, they are

i. Extraction
ii. Column chromatography
iii. Preparative separation
iv. Extraction:
v. Liquid –solid extraction
vi. Liquid-liquid extraction

1. Solid -Liquid extraction

Generally for isolation of impurity solvent selected for dissolve impurity, selected solvent must dissolve impurity but not solid matrix. But this method is not useful when solid matrix contain more than one impurity, at that time it is easy to use organic solvent for extraction. Because organic solvent have a unique property that is it volatized to get the impurity concentrated at low temperature.

Soxhlet Extraction

This is a popular for extraction of component of solid matrix. Natural products are extracted with suitable method of extraction using suitable solvent. In soxhlet extraction Continuous extraction of a component from a solid mixture. Boiling solvent vapors rise up through the larger side-arm Condensed drops of solvent fall into the porous cup, dissolving out the desired component from a solid mixture. When the smaller side-arm fills to overflowing, it initiates a siphoning action. The solvent, containing the dissolved component, is siphoned into the boiler below. Residual solvent then drains out of the porous cup, as fresh solvent drops continue to fall into the porous cup and the cycle repeats.

Steam Distillation

Steam distillation is special type of distillation process for temperature sensitive materials for example natural aromatic compounds. It is one of the popular method for purification of organic compounds, but from few days it has become obsolete by vacuum distillation. Separation by distillation is not possible at normal boiling point, that's why water or steam is introduced into the distillation apparatus. In condensation flask small amount of vaporized compound carries by water vapor, where the condensed liquids phase separate, for easy collection. For distillation at lower temperature this process is effective, reducing the deterioration of the desired products. After distillation the vapors are condensed as appropriate. Usually the immediate product is a Two-phase system of water and the organic distillate, allowing for separation of the components by partitioning or other suitable methods.

Supercritical Fluid Extraction

Supercritical Fluid Extraction (SFE) is the process of separating of two component that is one is extractant and another is matrix by using extracting solvent (supercritical fluid). Extraction is can be from solid or liquids mostly from solid matrix. SFE used as a sample preparation step for analytical purposes. SFE also used on a larger scale to either remove unwanted material from a product or collect a desired product. Most used supercritical fluid is carbon

dioxide4, sometimes co-solvents are used like ethanol, methanol. Extraction conditions for supercritical CO_2 are,
i. Above the critical temperature of 31°C and
ii. Above critical pressure of 74 bar.

Addition of modifiers may slightly alter this. This is only for CO_2, except where specified. The supercrical property can be vary with temperature and pressure like volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids.

Table 1: List of solvents for SFE[4]

Solvent	Pressure (ATM)	Temperature	Density (g/ml)
n-pentane	33.3	196.6	0.232
Carbon dioxide	72.9	-	0.448
Ammonia	111.3	132.3	0.24

2. Liquid –Liquid Extraction:
Liquid–liquid extraction also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid into another liquid phase. Liquid-liquid extraction is controlled by distribution or partition coefficient which defines the ratio of concentration of solute in two solvents a and b
Kd = Ca / Cb
Kd is the distribution co-efficient or partition coefficient.

Colum chromatography:
It is known that the rate of adsorption varies with a given adsorbent for different material. This principle of selective adsorption is used in column chromatography. This method used for separation of compound in both analytical and preparative application. Column chromatography is not only used for separation of components but also for purify substantial quantities of those components for analysis. Detection of eluent is done by uv spectrophotometry, either continuously by using a flow cell or periodically by monitoring the collected fractions from a given sample that alerts the emergence of uv active components. Commonly silica gel or alumina is used in classic adsorption chromatography. Ion exchange resins to chemically modified polydextran gels used primarily for the analysis of biological samples. For liquid-liquid partition chromatography columns inert carrier such as aqueous buffer or another polar solvent such as dimethyl foramide or dimethyl sulphoxide and elution is carried out with non-polar solvent.

Thin layer chromatography:
TLC is a form of liquid chromatography consisting of: a mobile phase (developing solvent) and a stationary phase (a plate or strip coated with a form of silica gel).Analysis is performed on a flat surface under atmospheric pressure and room temperature. The sample to be analyzed is added to the plate (which consist of a in a process called thin layer of silica gel or alumina coated on plastic or glass sheet) "spotting". If the sample is not already in solution, dissolve about 1 mg in a few drops of a volatile solvent such as hexanes, ethyl acetate, or methylene chloride. Place the prepared TLC plate in the developing chamber, cover the beaker with the watch glass, and leave it undisturbed on your bench top. Run until the solvent is about half a centimeter below the top of the plate. Quickly mark a line across the plate at the solvent front with a pencil. Allow the solvent to evaporate completely from the plate. If the spots are colored, simply mark them with a pencil. The RF value is calculated by measuring the distance the sample zone travels divided by the distance the developing solvent travels.
Rf = distance travelled by component / distance travelled by solvent

Gas Chromatography:
An analytical separations technique useful for separating volatile organic compounds, volatile by derivatization technique and the detector used should be non-destructive. Organic compounds separated due to differences in their participating behavior between the mobile gas phase and the stationary phase in the column.

High performance Liquid chromatography (HPLC):
The most widely used analytical separations technique utilizes a liquid mobile phase to separate components of mixture uses high pressure to push solvent through the column. Ideally suited for separation and identification of; amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, pharmaceuticals, pesticides, pigments, antibiotics, steroids, and a variety of other inorganic substances. Greater reproducibility is due to close control of the parameters affecting the efficiency of separation. In Reverse Phase Chromatography Stationary Phase is Nonpolar and Mobile Phase Polar. In Normal Phase Chromatography Stationary Phase is Polar and Mobile Phase is Nonpolar. The mobile phase are aqueous solutions containing methanol, water-miscible organic solvents also contain ionic species, in the form of a buffer solvent strength & selectivity are determined by kind and concentration of added ingredients ions in this phase compete with analyte ions for the active site in the packing. these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily. If more water added in mobile phase retention time can be increase. There by making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger.

High performance thin layer chromatography (HPTLC):
HPTLC method used for separation of the multi-component mixture.

- HPTLC- sophisticated and automated form of TLC.
- Powerful separation tool for quantitative analysis.
- Complimentary to other commonly used techniques.
- Simple separation techniques available to analyst.
- Considered as a time machine.

The substance is transported along by the mobile phase, resides for a certain time on the stationary phase, and is carried along and the components are separated due to two basic underlying principles of differences in affinity. During Adsorption the components dissolved in the mobile phase are adsorbed to the surface of silica / alumina. Partition results from the differences in the solubility of the substances in the mobile phases. The substances dissolved

in the mobile phase are partitioned between this phase and a second phase attached to a solid substrate, such as RP-silica or cellulose. Adsorption is suitable for separation of compounds differing in polarity while Partitioning is for separation of substances differing in their solubility. Mobile phase (driven by capillary action) travel over the surface of stationary phase and compounds with higher affinity to stationary phase travel slowly while the others travel faster. Separation of compound occur and spots are visualized this spots are individual compounds. Nature or characters are identified by means of suitable detection techniques.

Table No-2: Solvent Polarity

Solvent	Polarity	Solvent	Polarity
n-Hexane	0.1	Ethanol	4.3
Cyclohexane	0.2	Ethyl acetate	4.4
Carbon disulphide	0.3	Ethyl methyl ketone	4.7
Carbon tetrachloride	1.6	Dioxane	4.8
Isopropyl ether	2.4	Acetone	5.1
Toluene	2.4	methanol	5.1
Cholorobenzene	2.7	pyridine	5.3
Benzene	2.7	Acetonitrile	5.8
Diethyl ether	2.8	Acetic acid	6.0
Dichloromethane	3.1	Nitromethane	6.0
1,2 dichloroethane	3.5	Aniline	6.3
2 propanol	3.9	Ethylene glycol	6.9
Tetrahydrofuran	4.0	Dimethyl sulphoxide	7.2
chloroform	4.1	Water	10.2

Capillary Electrophoresis (CE):

This separation technique Separations based upon difference in charge to size ratios. The larger the ratio the faster an ion migrates in the electric field. Separations carried out in buffer filled capillary that is 10-100 µm i.d. & 40-100 cm length. Potential applied 10-30 K Volts. Separation based on differential rate of migration. The fused silica capillaries are used in CE are available in i.e. ranging from 10 to 100 µm. The capillary coated with polyimide.

$N=\mu e\ V/2D$

Where µe is the electrophoretic mobility, V is the applied potential D is the diffusion coefficient of the solute in cm^2s^{-1}.Capillary coatings: Cross-linked, linear poly--acrylamide (Si-C) C-8, C-18 Poly vinyl methyl siloxanediol Poly (methyl glutamate) aryl penta-fluoro group Epoxy coatings.

As impurity profiling plays a vital important role in pharmaceutical industries, researchers are working on same on different drugs few of them to understand the impurity profiling are as Aceclofenac[9], Artemether[10], Azithromycin tablets[11], Cephalexin[12], Clozapine[13], Ertapenem[14], Lumefantrine[15], Metformin Hydrochloride[16], Pravastatin[17], Rufinamide[18], Quetiapine[19], Sertindole[20], Silodosin[21], Zaleplon[22].

Characterization Methods

1. NMR
2. Mass spectroscopy
3. LC-MS
4. GC-MS

1. Nuclear Magnetic Resonance (NMR): NMR provides information about specific bonding between peak area and number of nuclei responsible for peak. Most important application of NMR is identification and structure elucidation of molecules. Analysis of multicomponent mixture.

2. Mass Spectroscopy (MS): Mass spectroscopy is a most accurate method for determining the molecular mass of the compound and its elemental composition. Mass spectroscopy is used to prove identity of two compound, establish the structure of new compound, give exact molecular mass, give molecular formula and most important for structure elucidation. Now a day's mass spectroscopy connected with various hyphenated techniques like GC-MS, LC-MS,LCMS-MS HPLC-DAD-MS, HPLCDAD-NMR-MS, Tandem Mass spectroscopy and capillary electrophoresis-Mass spectroscopy,.

3. **GC-MS:** To identify different substances within a test sample gas chromatography-mass spectrometry (GC-MS) method used, that combines the features of gas-liquid chromatography and mass spectroscopy. In this method gas chromatography separate volatile and semi-volatile compounds with great resolution. MS: can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. Sample vaporized by injection into a heated system, eluted through a column by inert gaseous mobile phase and detected. The sample is transported through the column by the flow of an inert, gaseous mobile phase, the carrier gas. Flow is regulated by the pressure regulators and gas metering valves. GC operates at atmospheric pressure and the MS ion source at 10-5 Torr.108 fold pressure difference. The carrier gas must be removed and GC peak components transferred to the MS ion source.

4. **LC-MS:** LC/MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. LC/MS became really popular with the introduction of the thermo spray interface and the particle beam interface. This is same as GC-MS but removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. For handle normal eluent flow rate 0.5-2.0ml $^{min-1}$ which is not handled by MS pumping system moving belt inlet systems, jet separators and vacuum nebulizers are used

Conclusion:

This study will help to a researcher who wants to work on impurity profiling. It gives idea about the impurity profiling, different types. This gives brief information about isolation and characterization, different methods of impurity detection with examples of the technique or drug.

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